Therapeutic Targets and Predictive Markers of Treatment Efficacy of Bladder Cancer

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Abstract: Bladder cancer (BC) is one of the most common types of cancers in the world. Despite various treatments are currently in place, the prognosis of BC does not seem to improve. Therefore, this prompts us to search for alternative treatments. The understanding of the molecular changes in cancer offers the prospect of targeting the key elements responsible for cancer development and progression, and thus, helps to develop a more effective and robust targeted therapy that does not endanger normal cells. At present, protein-based therapeutic targets such as programmed death ligand 1, epidermal growth factor receptor and aberrantly glycosylated integrin-α3β1, and genetic targets such as p53, human EGF receptor-2, and miR-23b are promising targets for BC treatment. In addition, predictive markers of treatment efficacy for BC, such as meiotic recombination 11, excision repair cross-complementing group 1, and multidrug resistance 1 gene are essential in facilitating clinicians to design and implement suitable and effective therapies for BC patients. This review discusses the pivotal role of these markers in the development of targeted therapy and the estimation of the clinical outcome of BC.

Keywords: Bladder cancer, Therapeutic targets, Therapeutic efficacy, Programmed death-ligand 1, Excisio repair cross-complementing group 1

1 Introduction

As the fourth most common cancer among men, bladder cancer (BC) is affecting about 430,000 people worldwide[1]. Urothelial carcinoma (mUC) accounts for more than 90% of all BC cases[2]. BC can be categorized into two types: Non-muscle invasive BC (NMIBC) and muscle-invasive BC (MIBC). NMIBC, accounting for about 80% of BC cases, does not invade the bladder walls. On the contrary, its non-muscle invasive counterpart, MIBC, which accounts for the rest of the BC cases, can metastasize and disseminate through the bladder wall, worsening the prognosis of BC[3].

In spite of the effective response of the main treatments of BC, including transurethral resection of bladder tumor, the prognosis of BC patients does not significantly improve[4]. Therefore, it is imperative to explore more effective therapies against BC. With the recent advances in next-
generation sequencing, our understanding of molecular changes underlying BC is growing[5]. These molecular markers in BC development, progression, and recurrence offer the prospect of specific targeted therapy in BC. Apart from that, certain molecular markers can also be used to predict treatment efficacy. Thus, molecular markers are becoming inseparable from their additional roles in improving treatments and clinical management of BC[2].

The aim of this review is to emphasize the current markers which serve as ideal candidates for the development of targeted therapy for BC. The mechanisms underlying targeted therapy based on candidate biomarkers will be introduced. Besides, several therapeutic targets that have been investigated in some pivotal clinical trials, and markers that may predict the efficacy of BC treatments are also described in this review. It is noteworthy that these markers have significant application values in the prediction of the sensitivity and specificity of treatments, the therapeutic response of treatments, and the prognosis of BC patients. The broadening impacts of therapeutic targets and predictive markers of treatment efficacy will prompt the development of more effective and rational BC treatments, thereby improving the clinical outcome of BC. These encouraging results further demonstrate the effectiveness of targeted therapy and highlight the requirement for precision therapy in BC.

2 Therapeutic targets

Targeted therapy is a treatment regimen targeting a known molecule or pathway that leads to tumorigenesis at the cellular and molecular levels. This molecule could be a protein or a gene fragment inside a tumor cell. Targeted therapy specifically targets the key factors that are responsible for tumor growth or progression, for instance, DNA damage, tumor microenvironment, angiogenesis, stress response, uncontrolled cell cycle, and apoptosis[5]. The interactions between targeted therapy and cancerous cells will activate the tumor suppressor genes while repressing the oncogenes, thereby generating the overall anti-tumor effects in the cancer environment[2]. Compared to conventional therapy, targeted therapy stands out for its targeted effects on cancerous cells without harming the normal cells; therefore, this therapy can limit the side effects on the body[6]. In BC, targeted therapy had been implemented for years and showed a positive response toward BC patients. Atezolizumab and erdafitinib, which target programmed cell death ligand 1 (PD-L1) pathway and fibroblast growth factor receptor 3 (FGFR3) pathway, respectively, are some of the drugs used in the targeted therapy[7,8]. The details of these markers for targeted therapy are discussed below.

2.1 PD-L1

Programmed death 1 (PD-1), also known as CD279, is a member of the CD28-B7 receptor family, which is mainly expressed on activated T lymphocytes. Its ligand, PD-L1, is a member of the B7 family. When PD-1 binds to PD-L1, it will cause T cell receptor-mediated lymphocyte proliferation and cytokine secretion[9,10]. PD-L1 is expressed in all cells, but is highly expressed in many tumor types[11-14]. Tumor cells can evade host-immune surveillance and clearance by expressing PD-L1 that binds to PD-1 to bring about immunosuppression in the tumor microenvironment. Therefore, the blockage of PD-1/PD-L1 signaling pathway activation can inhibit immunosuppression and thus, promote anti-tumor immunity[15,16].

As highlighted by the work of Tosuku Honjo, the 2018 Nobel Prize winner, the application of anti-PD-1 or anti-PD-L1 in cancer therapy has illuminated our understanding of harnessing the immune system to fight cancer[17]. MPDL3280A is a novel human immunoglobulin G1 monoclonal antibody that targets PD-L1 to attenuate the interactions between PD-1, PD-L1, and CD8[18]. Having a strong therapeutic activity against metastatic BC, the treatment of MPDL3280A demonstrated obvious responses in most BC patients. The immunohistochemical (IHC) level of PD-L1 was scored, and a higher IHC score indicates a higher expression level of PD-L1. The objective response rates (ORRs) of IHC 2/3 tumors were 43%, and the ORRs of IHC 0/1 tumor were 11%. These results suggested
that the higher the score, the higher the ORR of the MPDL3280A to the tumor. Although some patients showed physiological reactions such as decreased appetite and fatigue, the incidence of adverse events is lower than that of commonly used second-line treatment. The drug entered the Food and Drug Administration (FDA) approval process in June 2014.

In another study, Brower et al. reported the efficacy and safety of durvalumab, a PD-L1 targeting inhibitor, for the treatment of advanced bladder mUC[19]. A total of 61 advanced bladder urothelial patients received treatment, and finally, 42 patients (28 PD-L1 positive patients and 14 PD-L1 negative patients) were accepted for evaluation. Specifically, 13 out of 28 PD-L1 positive patients responded to durvalumab (response rate: 46.4%). However, none of 14 PD-L1 negative patients responded to durvalumab (response rate: 0.0%). Apart from that, common adverse reactions such as fatigue (13%), diarrhea (10%), and decreased appetite (8%) were observed during the treatment. The study showed that durvalumab is suitable for the treatment of PD-L1 positive BC patients, and the FDA approved durvalumab and its accompanying trials in May 2017. In addition, Apolo et al. identified the clinical effect of anti-PD-L1, avelumab, in the initial Phase Ib study on patients with metastatic mUC refracted to platinum[20]. All 44 patients had adverse reactions such as fatigue and nausea. The ORR was 18.2%, and the median overall survival (OS) was 13.7 months. FDA approved avelumab as a second-line treatment for local metastases or advanced UC.

Although immunotherapy targeting PD-L1 has enhanced the OS of different cancer types to some extent, it also faces some challenges. For instance, the evaluation of treatment response rate often requires the examination of PD-L1 expression levels in tumor cells and immune cells. Immunohistochemistry is the principal method for detecting PD-L1 expression. At present, however, there are no standardized quality control measures in administering the PD-L1 expression level test. Multiple kits that detect the expression of PD-L1 have been approved although their detection sensitivities are different. Nevertheless, their capability in distinguishing positive and negative samples is distinct. Take the case, we mentioned in the above, for example, for the treatment with durvalumab, among all PD-L1 positive patients, only 46.4% of patients responded to treatment, and 53.6% of patients did not respond to treatment. In other words, the therapeutic effect of the same antibody could still be different in different species even though they are similarly PD-L1 positive. Due to the difference in detection platform, antibody clone number, and tumor specimen, there is currently no unified cutoff value of PD-L1 positive expression[12,21,22]. Furthermore, the treatment effects on PD-L1-negative tumor are not prominent since it is only targeting PD-L1.

In addition to the difficulty of determining the test method and test standard, a study also found that binding affinity of the antibody to PD-L1 is also related to the therapeutic effects[23]. A high-affinity consensus “microbody” (HACmb), which has higher affinity for binding to PD-L1 than anti-PD-L1 antibodies, was developed. HACmb or an anti-mouse PD-L1 was administered intraperitoneally to mice when the tumor volume increased to 50 mm$^3$ or 150 mm$^3$. The results indicated that the anti-mouse PD-L1 only effectively inhibited tumor growth when the tumor volume was 50 mm$^3$, but did not control the tumor growth when the tumor volume reached 150 mm$^3$. Compared with the anti-mouse PD-L1, HACmb significantly suppressed the tumor growth when the tumor volume was either 50 mm$^3$ or 150 mm$^3$. Taken together, the therapeutic effects of PD-L1 based immunotherapeutics are also affected by its affinity, and the improvement of the binding affinity of antibodies to PD-L1 could help achieve a better treatment response. The affinity of anti-PD-L1 to PD-L1 expressed on tumors requires extensive research as patients experienced varying treatment responses. In addition to the challenges of detection methods and binding affinity, due to the complex regulatory network of tumors, the expression levels and mutations of certain genes can regulate the expression of PD-L1 and affect the efficacy of immunotherapy[24,25].

These studies emphasize the effectiveness of targeted therapy based on PD-1 or PD-L1
expression, and provide a reference for further research on biomarkers related to PD-1/PD-L1 pathway, demonstrating PD-1/PD-L1 pathway as an ideal target for developing immunotherapy for BC. However, some drawbacks and challenges limit the widespread application of immunotherapy in clinical treatment, and further research is needed to resolve these problems.

2.2 Epidermal growth factor receptor (EGFR)

EGFR is a large transmembrane glycoprotein with ligand-induced tyrosine-protein kinase activity. The binding of EGFR to epidermal growth factor (EGF) triggers multiple cascade signaling events, which in turn activate the related genes promoting cell division and proliferation. As one of the hallmarks of cancer, increased expression of EGFR has been found in a vast number of malignant tumors such as gastric cancer, squamous cell carcinoma of the head and neck, BC, and breast cancer. These related receptors include ErbB1/HER-1, ErbB2 (human EGF receptor-2 [HER2]/Neu), ErbB3/HER3, and ErbB4/HER4[26,27]. EGFR is involved in the regulation of most of the tyrosine kinase-based signaling pathways discovered to date, which triggers the key factor in the signaling pathways related to tumorigenesis, development, metastasis, and treatment resistance.

In one study, researchers used erlotinib, an EGFR inhibitor, to perform neoadjuvant chemotherapy on patients with T2 MIBC before surgery. The post-operative pathological analysis was performed to evaluate the complete response rate. Specifically, 25% of patients achieved a pathological complete response (stage T0), and 35% of patients had been clinically down-staged (stage ≤T1)[28]. During the 24.8-month follow-up, half of the patients were still alive, demonstrating that the use of erlotinib for neoadjuvant therapy is clinically beneficial in the short term.

Cetuximab can bind to the extracellular domain of EGFR and be used as an anti-EGFR drug. In a clinical trial of cetuximab for the treatment of BC, the patients receiving cetuximab monotherapy had disease progression within 8 weeks after treatment, so the monotherapy experiment was suspended ultimately[29]. A previous study reported that patients treated with paclitaxel monotherapy had a progression-free survival (PFS) of 2 – 3 months[30]. In this experiment, 12 of 28 patients who received a combination of cetuximab and paclitaxel did not progress at the end of follow-up at week 16. Although the antitumor effect of cetuximab is not apparent when used alone, the combined use with paclitaxel can increase the antitumor effect of paclitaxel. From the above results, EGFR inhibitors exert beneficial therapeutic effects regardless of how this class of drugs is taken, either in monotherapy or combination therapy mode.

Moreover, a study revealed that leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1) can suppress BC through the negative regulation of EGFR. Chang et al. used various molecular techniques such as reverse transcription-polymerase chain reaction, Western blot, and co-immunoprecipitation to study the effects of LRIG1 gene on EGFR in BC cell lines T24 and 5637. The results demonstrated that a negative correlation between LRIG1 and EGFR expressions in BC. Furthermore, the EGFR/LRIG1 ratio was significantly higher in BC tissues than that of normal tissues[31]. As for the principle of this interaction, a study showed that the ectopic expression of E3 ubiquitin ligase c-Cbl in cells can accelerate the degradation of ErbB-1[32], and the introduction of LRIG1 played a role similar to c-Cbl, so LRIG1 “negatively regulate” the expression of EGFR by ubiquitination-based degradation. Therefore, inhibiting the expression of EGFR by upregulating the expression of LRIG1 can effectively inhibit tumor progression, which can become a therapeutic strategy. Further experiments show that the upregulation of LRIG1 can induce apoptosis, inhibit the growth of tumor cells, and enhance the sensitivity of tumor cells to chemotherapy drugs. The overexpression of LRIG1 through adenovirus vector results in inactivation of the EGFR pathway, decreased B-cell lymphoma-2 (Bcl-2) expression, and induced Bax expression, which is helpful for apoptosis[33]. Yan et al. also showed that the aforementioned events culminate in increased sensitivity to cisplatin treatment in T24 cell line.
In summary, the negative regulation of EGFR by LRIG1 is a promising target for developing BC therapy.

2.3 Aberrantly glycosylated integrin α3β1 (AG-α3β1)

Integrins are a family of transmembrane heterodimeric proteins, composed of α and β subunits, and can be used as receptors for laminin, collagen, and other components in the extracellular matrix. For example, integrin can interact with collagen on the basement membrane to form a complex, connecting the extracellular matrix with the intracellular skeletal network. This cell adhesion event is orchestrated by integrin. The destruction of this complex will help the cells migrate and make the cells more invasive, leading to an increased likelihood of metastasis[34].

The AG-α3β1 is a BC marker discovered by Li et al. who found that BCMab1, the anti-human BC antibody, recognized, and ligated to AG-α3β1 in the cytological experiment[35]. This engagement inhibited the proliferation, adhesion, invasion, and metastasis of bladder tumor cells. BCMab1 can also mediate phagocytic killing of tumors by macrophages and NK cells through antibody-dependent cellular phagocytosis and antibody-dependent cell-mediated cytotoxicity, respectively. In vivo experiments showed that intravesical administration of BCMab1 significantly inhibited the growth of orthotopic tumors in nude mice with no local or systemic side effect observed in nude mice[35]. This study proved that the BCMab1 antibody has a strong ability to suppress bladder tumor growth with minimal side effects. Meanwhile, its epitope AG-α3β1 is expected to become a new therapeutic target for BC. BCMab1-Ra is an antibody-based drug produced by combining BCMab1 with ricin A chain (Ra)[36]. In this study, a patient with multiple BC was administered with BCMab1-Ra treatment for 20 weeks. The cystoscopy results showed that the tumor disappeared 26 weeks later. No side effects and hematuria caused by the drug were found throughout the treatment duration[36].

BC stem cells (BCSCs) are considered the key to the occurrence and recurrence of BC. Li et al. used a combination of BCMab1 antibody and CD44 antibody to isolate BCMab1 CD44+ cell subpopulation as BCSCs with stem cell-like properties, and proved that the Hedgehog signaling pathway was activated in this subpopulation[37]. In addition, the same study also showed that galactosyltransferase I (GALNT1) can regulate the SHH signaling pathway by mediating the O-link glycosylation of SHH protein to maintain the self-renewal capacity of BCSCs and promote the occurrence and development of bladder tumors. Infusion of GALNT1 siRNA and SHH-targeted inhibitors can effectively inhibit the occurrence and development of bladder tumors and is expected to become a new strategy for the prevention and treatment of BC. Since BCMab1 is a strong candidate for the development of targeted therapy in BC, further research is needed to translate the preclinical findings into clinical use.

2.4 Other therapeutic targets

Apart from the markers discussed above, many other therapeutic targets have been documented. These include vascular endothelial growth factor (VEGF), FGFR3, miR-133a, lnc RNA, and urothelial carcinoma associated 1 (UCA1). Among them, therapy targeting different gene loci is one of the most promising BC treatments. At present, common gene targets which are widely studied are p53, p21, p16, Bcl-2, HER2, miR-23b, and PIK-3[38-41].

HER2 is a well-studied proto-oncogene of breast cancer, and its overexpression is related to tumorigenesis and invasion[42]. Amplification or overexpression of HER2 was also found in invasive BC, but the exact ratio of BC patients possessed this alteration remained unclear[43-45]. In an experiment using immunohistochemistry and fluorescence in situ hybridization, the overexpression of HER2 gene was detected in 9.2% of BC tumor specimens, and more specifically, the HER2 gene amplification was detected in 5.1% of MIBC specimens, suggesting that HER2 is a potential therapeutic target for patients with locally progressive or metastatic BC who are positive for HER2 expression[39].

MicroRNA (miRNA) is a non-coding RNA. It has been reported that the imbalance of miRNA is related to the progression of BC. For
example, the expression of tumor-suppressive miR-133a was decreased in BC compared with peritumor tissues, resulting in an increase of the expression of its target gene, i.e., glutathione S-transferase $\pi$ (GSTP1)\textsuperscript{[46]}. The anti-apoptotic effect mediated by GSTP1 promoted the tumorigenesis of BC. The anti-apoptotic effect mediated by GSTP1 is maintained in human BC\textsuperscript{[46]}. Another study by Majid et al. found that $miR$-$23b$ was downregulated in BC cell lines and tumor tissues. Interestingly, the patient group with high $miR$-$23b$ expression had higher OS\textsuperscript{[47]}. Further, in vitro experiments showed that $miR$-$23b$ overexpression in BC cell lines inhibited cell proliferation, destructed colony formation and inhibited tumor invasiveness properties. Luciferase reporter assay indicated that Zeb1, the key molecule in the regulation of epithelial-mesenchymal transition, is the target of $miR$-$23b$. The knockdown of $miR$-$23b$ promoted invasion and migration of BC. It is the first demonstration that $miR$-$23b$ is a potential biomarker and tumor suppressor for BC and directly targets the oncogene Zeb1. Therefore, it is feasible to deduce that the enhancement of $miR$-$23b$ expression can be exploited for developing BC targeted therapy.

### 3 Predictive markers of treatment efficacy

For MIBC patients who are unable to tolerate or unwilling to undergo radical cystectomy, radiotherapy, and chemotherapy can be used as alternative treatments. However, not all patients are sensitive to radiotherapy or chemotherapy. Giving treatment blindly often delays optimal timing of surgery, and even some patients will have side effects. Hence, the difficulty in delineating the sensitivity of patients to radiotherapy and chemotherapy remains the main issue in predicting the treatment efficacy in BC patients.

The main principle of radiotherapy and chemotherapy is to cause DNA damage to cells and cause cell death. The resistance of patients to this treatment is mainly due to the abnormal DNA repair mechanism initiated in tumor cells. Therefore, the expression level of some genes related to DNA repair such as meiotic recombination 11 (MRE11), and excision repair cross-complementary group 1 (ERCC1) is closely related to the prognosis of patients, and can be used as a marker to predict the prognosis level after radiotherapy and chemotherapy. In addition, multidrug resistance gene 1 (MDRI), a well-known drug resistance gene, is also a predictive marker of treatment efficacy.

#### 3.1 MRE11

MRE11 is an important component of MRE11-RAD50-NBS1 (MRN) complex. It can recognize and repair DNA double-strand breaks. In recent years, many researchers emphasized that MRE11 can be used as a predictive marker of the efficacy of BC radiotherapy\textsuperscript{[48]}. In a study, researchers used immunohistochemistry to examine the expression of MRE11, RAD50, NBS1, ATM, and H2AX in bladder tumor specimens derived from 86 patients who had undergone radiotherapy group and 88 patients who had undergone cystectomy\textsuperscript{[49]}. The results showed that in the radiotherapy group, patients with low MRE11 expression had lower tumor-specific survival rates as compared to those with high expression (43.1% vs. 68.7%). This was confirmed in the radiotherapy validation cohort (43.0% vs. 71.2%). In contrast, there was no significant correlation between MRE11 expression and tumor-specific survival in the cystectomy group. In another study, the researchers measured the expression level of MRE11 protein in a cohort of patients undergoing radiotherapy by immunohistochemistry and evaluated the patient’s disease-specific survival (DSS)\textsuperscript{[50]}. The results showed that the high expression level of MRE11 and long DSS is significantly correlated. In summary, these findings indicate that MRE11 expression can be used as a predictive marker of efficacy in guiding physicians to select the best treatment regimen for patients with the aim of improving overall clinical outcome.

Teo et al. used second-generation gene sequencing technology to evaluate the correlation of MRE11A gene variants and single-nucleotide polymorphisms (SNPs) with the efficacy of radiotherapy in MIBC patients\textsuperscript{[51]}. The results showed that prognosis was significantly poorer when the patient carried at least one of the six
MRE11A gene variants. In addition, there was a negative correlation between SNP rs1805363 and tumor-specific survival rate. However, there was no significant association between MRE11A gene polymorphism and prognosis in cystectomy patients. This study suggested that the detection of MRE11A gene variants and polymorphisms by second-generation gene sequencing can be used to predict the efficacy of radiotherapy in patients with MIBC. Further validation concluded that the detection of SNPs in MRE11A gene will provide an effective basis for the treatment selection of MIBC patients, maximizing radiotherapy benefits of patients.

3.2 ERCC1

ERCC1 is the main component of the nucleotide excision repair system. ERCC1 can form a dimer with XPF to remove damaged and mismatched DNA, and then connect the excised fragments to repair DNA; so, ERCC1 plays an important role in maintaining DNA stability [52]. There have been many reports that the expression level of ERCC1 is related to the prognosis of chemotherapy or radiotherapy. Investigators used four human high-grade BC cell lines: 5637, T24, C18-2 (multidrug-resistant subline of T24), and CDDP10-3 (cisplatin-resistant subline of T24) to investigate the relationship between ERCC1 expression and cell growth inhibition induced by cisplatin-based chemotherapy and radiotherapy [53]. The results showed that ERCC1 expression level in C18-2 cells was 5.96-fold higher than in T24 cells. In contrast, C18-2 and CDDP10-3 showed greater resistance to radiotherapy and chemotherapy than T24 cells. After suppressing the ERCC1 through siRNA knockdown, C18-2 and CDDP10-3 cells remained resistant to radiotherapy, but became sensitive to chemotherapy. In addition, the study also conducted a clinical sample analysis in which 75% of the ERCC1-positive patients showed a non-complete response to chemoradiation therapy (CRT), whereas 85.7% of their ERCC1-negative counterparts showed complete response to CRT [53].

In a study that analyzed the expression levels of ERCC1, breast cancer 1 (BRCA1), ribonucleotide reductase M1 (RRM1), and caveolin-1 mRNA after gemcitabine/cisplatin/paclitaxel or gemcitabine/cisplatin (GC), it was found that the patients with low ERCC1 had a higher median survival (25.4 months) than patients with high ERCC1 expression levels (15.4 months), and the expression levels of BRCA1, RRM1, and caveolin-1 had no significant correlation with survival rate, indicating that ERCC1 may predict the therapeutic effect of platinum drugs in BC patients [54]. Sun et al. evaluated the prognostic value of ERCC1 in BC patients undergoing neoadjuvant GC chemotherapy [55]. It was found that the 5-year OS rate of ERCC1-positive patients was 41.6%, while the 5-year OS rate of ERCC1-negative patients was 71.8%. The expression of ERCC1 was significantly associated with shorter survival time (hazard ratio: 2.64), indicating a correlation between ERCC1 expression level and BC prognosis. These findings suggested that ERCC1 expression level can be used to predict efficacy of radiotherapy and chemotherapy in patients with BC.

3.3 MDR1

MDR1 is located on the long arm of human chromosome 7 (7q21). The protein expressed by the MDR1 gene is P-glycoprotein (P-gp), which transports drugs out of the cell, making the cell drug-resistant. Some reports have shown that the expression of MDR1 is also related to the progression and drug resistance of BC. A study showed that MDR1 is also expressed in normal urethral epithelial tissues, indicating that it may play a role in protecting epithelial tissues from various local and systemic toxins [56]. However, in low-grade BC, MDR1 mRNA levels are 6 times lower than in normal tissues, while in high-grade BC, MDR1 mRNA levels are 2 times higher than in low-grade BC. Another study found that after doxorubicin (DOX) treatment, the expression levels of MDR1 in tumor cells that are resistant to treatment and tumor cells that relapse after chemotherapy are higher than those of the primary tumor [57]. These findings prove that MDR1 expression is related to the progression and drug resistance of BC and relapse after treatment. Therefore, MDR1 can be used as a marker to predict the prognosis of chemotherapy in patients with BC.
Cheng et al. found that in the presence of DOX, transferring P-gp into drug-sensitive cells can make the cells less likely to be killed by drugs, the drug resistance index (RI) increases, the expression level of MDR1 mRNA increases, and the cells become stable and resistant[58]. Another study analyzed the correlation of the expression levels of MDR1, MRP1, and six human multidrug resistance-associated protein subfamily members (MRP2-7) that are structurally similar to MRP1 with DOX resistance[59]. Relative to the patients with untreated primary tumors, 64% of patients with residual tumors had a 5-fold increase in MDR1 expression after systemic DOX chemotherapy. Among the 17 tumors that relapsed after chemotherapy, the expression levels of MDR1, MRP1, MRP2, and MRP3 in 65 – 94% of tumors were higher than those in primary tumors. This proved that the expression levels of MDR1, MRP1, MRP2, and MRP3 mRNA were related to DOX resistance.

Hoffmann et al. found that the expression of MDR1 and ERCC1 is independent factors associated with overall PFS in MIBC patients, and patients with high MDR1 expression were less sensitive to the treatment regimen consisted of methotrexate, vinblastine, epirubicin, and cisplatin (MVEC) combination, and patients with high ERCC1 expression were less sensitive to treatment regimen consisted of cisplatin and methotrexate (CM) and MVEC regimen[60]. Thus, MDR1 and ERCC1 can be used as predictive markers of efficacy of neoadjuvant chemotherapy in locally advanced BC.

### 3.4 Other predictive markers

Apart from the markers discussed above, many other markers for prediction are documented. Ataxia-telangiectasia mutated (ATM) is a protein of phosphatidylinositol 3-kinase-like protein kinases family. It phosphorylates and activates downstream substrates and then recruits DNA repair-related complexes to repair DNA breaks[61]. Retinoblastoma 1 (RB1) is a tumor suppressor protein that can regulate the transition of the cell cycle from G1 to S phase[62]. Hence, the loss of function of ATM or RB1 protein may lead to a reduction in double-strand break repair response and uncontrolled cell proliferation. This leads to an increased sensitivity and susceptibility of tumor cells to radiation therapy or cytotoxic drugs[63]. For example, human disabled homolog 2 interaction protein (DAB2IP) is a tumor suppressor gene, and its downregulated expression level may confer BC cell resistance to ionizing radiation (IR). In one study, the cells from 5637 cell line were transfected with siRNA to inhibit the expression of DAB2IP and ATM[64]. In subsequent irradiation experiments, the surviving fractions at 2 Gy (SF2) of control cells were 0.38 ± 0.07. The SF2 of siDAB2IP cells was 0.69 ± 0.06. When the expression of ATM in siDAB2IP cells was suppressed, the SF2 value decreased to 0.37 ± 0.08, indicating that knockdown of DAB2IP rendered the cells resistant to IR but the knockdown of ATM can restore the sensitivity of cells to IR. Fanconi anemia complementation Group C (FANCC) belongs to the FA/BRCA network that recognizes and repairs DNA damage. Mutations in certain genes in the network are closely related to tumorigenesis. FA-BRCA network silence can make cells highly sensitive to cisplatin[65].

In a prospective study, Plimack et al. collected pre-treatment tumor tissue specimens from patients and treated with cisplatin-based neoadjuvant chemotherapy regimen[66]. Subsequently, 287 cancer-related genes in tumor tissue were sequenced, and base substitutions, copy number changes, and chromosome rearrangements were analyzed. The results showed that the DNA repair gene alterations were significantly more abundant in patients who fully responded to cisplatin-based neoadjuvant chemotherapy compared with drug-resistant patients. Specifically, three DNA repair genes were detected, including ATM, RB1, and FANCC. Gene alterations including stop, insertion/deletion, splicing, amplification, loss, missense, and other harmful alteration to protein function, will be used as predictive indicators. For example, missense mutations of ATM occur at K2413Q and Y2009H; stop mutations of RB1 at R787* and F216fs*7; and splice mutations of RB1 at linker 920. The sensitivity and specificity of using these gene alterations as the markers to predict the
patient’s response to chemotherapy were 87% and 100%, respectively. These alterations were significantly correlated with better PFS and OS of BC patients.

In the updated follow-up results (74 months), the 5-year survival rate of patients with at least one mutation was 85%, while the 4-year survival rate of patients without mutations was 45%[67]. These results indicated that the alterations in ATM, RB1, and FANCC render bladder tumor patients increased sensitivity to cisplatin and can be used as markers to predict response of MIBC to cisplatin-based chemotherapy regimen.

4 Conclusion

Accompanied by the recent progress in tumor genetic analysis, years of rigorous investigation and exploration have more precisely delineated the molecular underpinnings of BC. These molecular changes are responsible for the activation of tumorigenesis while interrupting the tumor-suppressing pathways. Therefore, the design of agonists and antagonists of these molecular markers could be an attractive idea for the development of targeted therapy. Besides, the in-depth understanding of the molecular markers in BC regulation could help in predicting therapeutic efficacy. Although a variety of therapeutic targets and predictive markers for treatment efficacy have been recorded, most of them are still in the experimental stage, and a large number of clinical trials have not been conducted or the number of samples included in the evaluation is insufficient. Thus, the validity of these targets and markers is still questionable. It is still necessary to evaluate more samples and conduct appropriate clinical trials to verify the accuracy of the predictive markers. Besides, as described in this review, the determination of the cutoff value for the positive expression of different patients is also worth studying, which is very important for the development of individualized treatment. In addition, although the corresponding antibody and other therapeutic strategies have been developed for different markers, optimization methods such as increasing antibody affinity are still needed to improve the therapeutic effect. Hence, more clinical trials could facilitate the translation of basic science into clinical applications, including the conception of novel targeted therapies against BC.

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Conflicts of interest

The authors declare no potential conflicts of interest.

Author contributions

Z.Y. and C.L. conceived the idea of this review. Z.Y. and M.S. wrote the paper. Z.Y., M.S. and L.W. revised the paper.

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